

Oral verrucous hyperplasia versus oral verrucous carcinoma: A clinicopathologic dilemma revisited using p53 as immunohistochemical marker

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Abstract

Background: Oral verrucous hyperplasia (OVH) and oral verrucous carcinoma (OVC) are two distinct clinicopathologic verrucous lesions. However, the distinction between the two lesions still remains enigmatic. It is almost impossible to distinguish them clinically. Thus, the final diagnosis rests on the histopathological characteristics of both lesions, being distinguished from each other by an exophytic and endophytic growth pattern, respectively.

Methods: This institutional study was planned to review retrospectively two series of patients with histologic diagnoses of VH ($n = 27$) and VC ($n = 27$) to investigate their clinicopathological features and to analyze the role of immunohistochemical (IHC) marker p53 protein in distinguishing between the two verrucous lesions. The biopsies of the histopathologically diagnosed cases spanning last 10 years were retrieved from the archives of the Oral Pathology department of the institution. Clinical data were tabulated and analyzed for age, gender, site and tobacco habits. IHC staining was done on all the samples using p53 antibody.

Results: Applying Chi-square test, the buccal mucosa was the most common affected site and tobacco chewing was more prevalent habit in both these lesions ($P > 0.05$). While the elderly males (>60 years) were the most commonly affected group in VC, a relatively younger age group of males (30–39 years) was more commonly affected in VH ($P < 0.05$). IHC staining with p53 antibody did not show any significant difference between these two verrucous lesions ($P > 0.05$).

Conclusion: VH and VC are closely related lesions distinguished by an adequate biopsy sample.

Key Words: p53 protein, tobacco chewing, verrucous carcinoma, verrucous hyperplasia

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INTRODUCTION

Oral verrucous hyperplasia (OVH) may be a precursor lesion of oral verrucous carcinoma (OVC) and it resembles oral VC both clinically and histopathologically.^[1] OVC is a rare variant

of oral squamous cell carcinoma (OSCC), first described by Ackermann, and henceforth also known as Ackermann's tumor.^[2] Despite differences, a close relationship also exists between VC and OSCC. VH and VC often coexist with dysplasia and OSCC,

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and VH and VC have been reported to progress into OSCC.^[3,4] OVC has unique histopathologic features and it is extremely challenging to diagnose it accurately. For correct evaluation of these two lesions, an adequate biopsy sample is of utmost importance along with an interaction between the clinician and the pathologist. In fact, some studies have shown that it took an average of three to four biopsies before a correct diagnosis of OVH or OVC could be made.^[5,6] Shear and Pindborg^[3] described the histopathological differences to differentiate between these two verrucous lesions. The hyperplastic epithelium was superficial to adjacent normal epithelium in VH; however, in VC, there was a pushing-border invasion of the hyperplastic epithelium into the underlying connective tissue. Nevertheless, differential diagnosis of these verrucous lesions remains an enigma for the histopathologists either because of the lack of adjacent normal epithelium when a biopsy is performed or because of the improper orientation of the specimen.

p53 is a tumor suppressor gene which is considered as the guardian of the genome. The loss of p53 function early in the carcinogenic process may contribute to the increase in the genetically altered cells resulting from exposure to carcinogens.^[7] Various immunohistochemical (IHC) studies have exhibited p53 staining in oral premalignant lesions and thus p53 aberration is a common early event in oral carcinogenesis.^[8-10] Numerous studies have been performed on the biopsies of OSCC demonstrating overexpression of p53 protein.^[11,12] To date, very few studies of the p53 protein expression have been performed simultaneously on VH and VC, to help in differentiating the two lesions. Therefore, we planned this institution-based retrospective study to analyze the significant clinicopathological differences between these two oral verrucous lesions. In addition, the objective of the study was to use an IHC technique to examine the expression of p53 protein in oral VC and oral VH biopsies, thus assessing its role in differentiating oral VH from oral VC histopathologically.

MATERIALS AND METHODS

Samples

The present study was a retrospective study where the clinical details of the patients such as age, sex, habits, site of the lesion, clinical diagnosis and histopathology of the lesion were retrieved and recorded from the archives of the oral and maxillofacial pathology department of the institution over a span of 10 years (from January 1, 2004 to May 31, 2014). After obtaining formalin-fixed, paraffin-embedded specimens, serial sectioning was done and the first section was stained with hematoxylin and eosin for histopathological assessment to reach a consensus with the original diagnosis. The diagnoses of oral VH and oral VC were based on the histological examination of hematoxylin- and eosin-stained tissue sections.

The histological criteria for the diagnosis of oral VC included: (a) Epithelial overgrowth with wide and elongated rete ridges exhibiting a pushing-border invasion into the underlying connective tissue, (b) papillary or verruciform epithelial projections with abundant parakeratin production and (c) the lesional epithelial cells showing a normal maturation pattern with no significant degree of cellular atypia^[13] [Figure 1].

While the histological criteria employed for the diagnosis of oral VH were: (a) Epithelial hyperplasia with parakeratosis or hyperkeratosis and verrucous surface, (b) no invasion of the hyperplastic epithelium into the lamina propria compared with adjacent normal mucosal epithelium^[13] [Figure 2].

The detailed demographic and clinical data of oral VH and VC patients were retrieved from the clinical records of the patients and were analyzed in a tabulated form as listed in Table 1. Clinical details recorded from the patient's records were age, sex, anatomical site, clinical features, clinical diagnosis and tobacco as well as alcohol habits. Details of the duration and frequency of habit could not be recorded because of the retrospective nature of the study. Histopathological features of oral VH were recorded in a tabulated form listed under the following headings: The verrucous projections, the presence of keratinization (ortho and parakeratinization); degrees of epithelial dysplasia; the thickness of stratum spinosum; the width of the rete ridges.

Immunohistochemistry

Serial sections of 4- μ m thickness were cut from all the retrieved samples and IHC staining was performed using a peroxidase-labeled streptavidin-biotin technique. The tissue sections were heated in a plastic slide holder (DAKO, Copenhagen, Denmark) containing 0.01 M citrate buffer in a

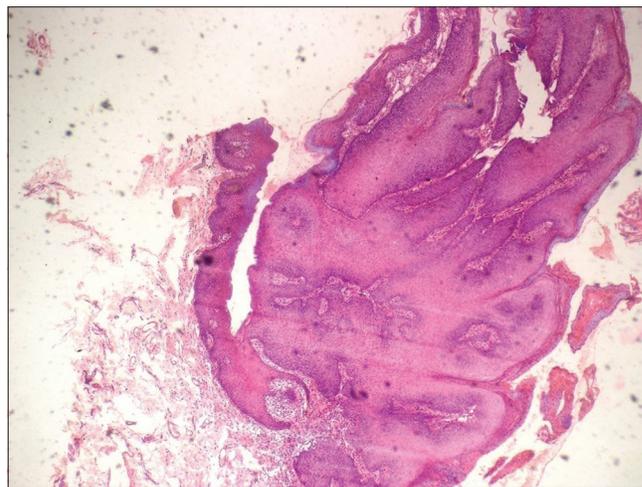


Figure 1: Photomicrograph of oral verrucous carcinoma. Tumor growth is mainly endophytic with broad pushing margins relative to the normal location of the oral epithelium (H&E stain, $\times 40$)

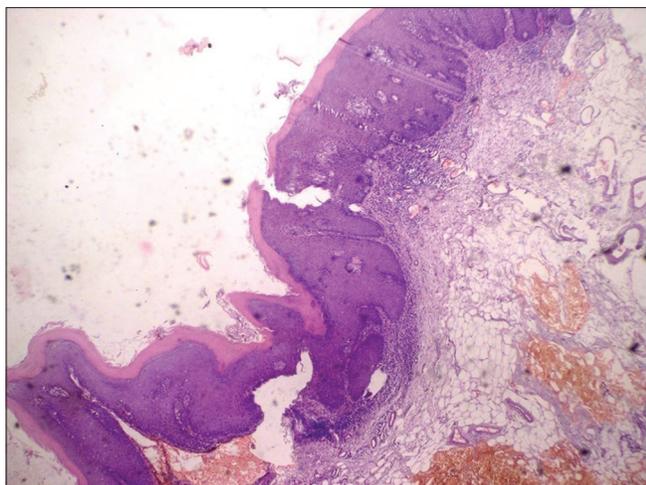


Figure 2: Oral verrucous hyperplasia shows both sharp and blunt verrucous projections with adjacent normal epithelium showing rete ridges at the same level (H&E stain, $\times 100$)

microwave oven for 12 min (3 min for each cycle) for antigen retrieval. Next, the sections were treated with 3% H_2O_2 in methanol for 10 min to quench endogenous peroxidase activity. After washing in 10 mM Tris-buffered saline (TBS), pH 7.6, sections were incubated with 10% normal goat serum to block nonspecific binding. Sections were then incubated for one hour, with the primary antibody, i.e. p53 protein. Again, sections were washed in TBS and treated with a mixture of biotinylated goat anti-mouse immunoglobulin G (Ig G) and biotinylated goat anti-rabbit IgG, and subsequently, with the streptavidin-peroxidase conjugate. The 0.02% diaminobenzidine hydrochloride containing 0.03% H_2O_2 was used as a chromogen to visualize the peroxidase activity. The sections were counterstained with Harris' hematoxylin and mounted in disterene dibutylphthalate xylene (DPX). Tissue sections of OSCC were taken as positive controls and were considered positive for p53 only when distinct nuclear staining was identified, while gingivectomy tissues, obtained after patient's written consent, served as the negative control, which were provided by the department of periodontology of the institution.

The positive samples for p53 protein were determined by examining sections at $\times 400$. A specimen with $\geq 10\%$ positive nuclei by counting all basal and parabasal cells in the lesion was defined as positive similar to the criteria by Wong *et al.*^[11] According to this criteria, cells of tumor nests were counted along with the number of p53 immunoreactive cells in OSCC samples, thus calculating the aggregate positive cells.

Statistics

The detailed comprehensive data of clinical details of both series of patients as well as the comparative positivity of p53

Table 1: Comparison of clinical and demographic data of OVH and OVC patients (from 2004-2014) over a span of 10 years

Characteristic parameter	OVH (n=27)	OVC (n=27)	χ^2 value Chi square test	P
Age (years)				
Mean (SD)	46.3 (5.58)	61.1 (6.58)	11.57	0.003
20-39	11	3		Significant ($P < 0.05$)
40-60	10	6		
>60	6	18		
Gender				
Female	5	5		
Male	22	22		
Site				
Tongue	3	0		
BM	16	22		0.2617
Gingiva	1	3	8.876	NS
Upper lip	1	1		$P > 0.05$
Lower lip	4	1		
Palate	1	0		
FOM	1	0		
Adverse habit				
Smoking	9	17		
Smokeless	18	20	1.056	0.5899
Both	8	10		NS
Lesion size (cm)				
Mean (SD)	2.9 (14.3)	4.2 (18.5)		
<2	15	4	10.968	0.0042
2 - 4	10	15		Significant
>4	2	8		

BM: Buccal mucosa, FOM: Floor of mouth, NS: Non significant, SD: Standard deviation

protein in both series of patients were analyzed with Chi-square test.

RESULTS

This study comprehensively analyzed the clinical records of the histopathologically diagnosed cases of OVC and OVH [Table 1]. The evaluation of oral VH patients showed a preponderance of the relatively younger age group, i.e., in the 3rd decade followed closely by patients in the 5th and 6th decade. The mean age of our patients with OVC was significantly older than that of patients with oral VH ($P < 0.05$) and the mean tumor size of oral VC lesions was significantly larger than that of oral VH lesions ($P < 0.05$). However, no significant differences in the other clinical parameters including the gender of the patient, lesion site and tobacco habits were found between the two series of patients.

On clinical examination, some lesions extended to adjacent sides, and the area most extensively involved was recorded. The buccal mucosa was the most frequently involved site observed predominantly in males in both the lesions and no significant difference was found between the two groups ($P > 0.05$). The gingiva and palate were the other common sites in VC while lower lip was the next most commonly involved site in VH.

Clinically, two patterns were observed in VH, namely, sharp and blunt. Both were observed in our patients. The sharp variety comprised long, narrow, heavily keratinized verrucous processes. The second variety was the blunt variety and it exhibited verrucous processes that were broader and flatter and not heavily keratinized. However, in both types, leukoplakic areas may also be a part of the lesion in the same patient. Histopathologically, both varieties were observed compatible with the clinical variations. The sharp variety of VH showed long and narrow verrucous projections which were heavily keratinized [Figure 3]. Shorter and broader exophytic projections which were not heavily keratinized were observed in the blunt pattern of VH [Figure 4]. Hyperorthokeratosis was predominantly seen in the sharp variety whereas the blunt pattern exhibited mostly parakeratosis. In most of the cases, epithelial hyperplasia was observed as an increase in the thickness of stratum spinosum [Table 2]. When the thickness of the epithelium was normal but showed verrucous projections, it was reported as VH. In the majority of VH cases, rete ridges were broad and blunt [Table 2].

Dense and diffusely spread chronic inflammatory cell infiltration was present in the lamina propria in most of the cases of VH and VC, comprising mainly of lymphocytes, plasma cells and histiocytes. Epithelial dysplasia was a more prominent feature in VH and was graded ranging between mild and severe [Table 3].

No positive staining for p53 protein was observed in control normal gingival epithelium. Distributions of the immunostaining positive cells were similar for both OVC [Figure 5a and b] and OVH [Figure 6a and b] samples. The positive-staining cells were mainly located in the basal and parabasal layers for the p53 protein. Few sections also showed irregular moderate staining within the basal and parabasal layers but with $\geq 25\%$ positive cells. No significant association of IHC marker in OVC or OVH samples with patient's age, lesion location,

tumor size and oral habits of the patient was found in the present study. There were no specimens that exhibited diffuse p53 immunoreactivity through all the layers of the epithelium. Eighteen specimens of OVH and 21 specimens of OVC defined as negative for p53 immunoreactivity showed either the complete absence of p53-reactive cells or only scattered, sparsely located p53-reactive cells in the basal cell layer. Comparative evaluation of the P53 stained sections in both groups was done

Table 2 : Histologic characteristics of VH (n=27)

Histologic features	No. of cases (n=27)
Verrucous projections	
Sharp	8
Blunt	17
Both	2
Keratinization	
Parakeratinized	20
Orthokeratinized	6
Both	1
Thickness of St. spinosum	
+	3
++	10
+++	14
Width of rete ridges	
Broad	23
Narrow	4
Both	6

Table 3: Comparison of histologic features of epithelial dysplasia and p53 immunoreactivity between OVH and OVC

Histologic characteristics	VH (n=27)	VC (n=27)	Chi-square test	
Epithelial dysplasia				
Mild	14 (51.8%)			
Moderate	4 (14.8%)			
Severe	3 (11.1%)			
Hyperplasia	6 (22.2%)			
IHC marker p53 positivity	9	6	0.8308	0.3621 NS

NS: Not significant

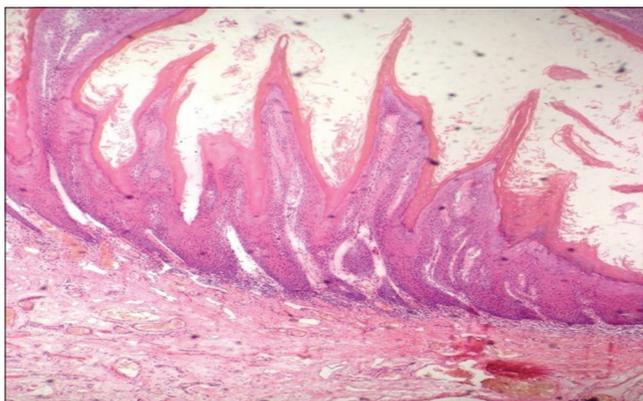


Figure 3: Photomicrograph of a verrucous hyperplasia. The verrucous growth is exophytic with sharp projections (H&E stain, $\times 100$)

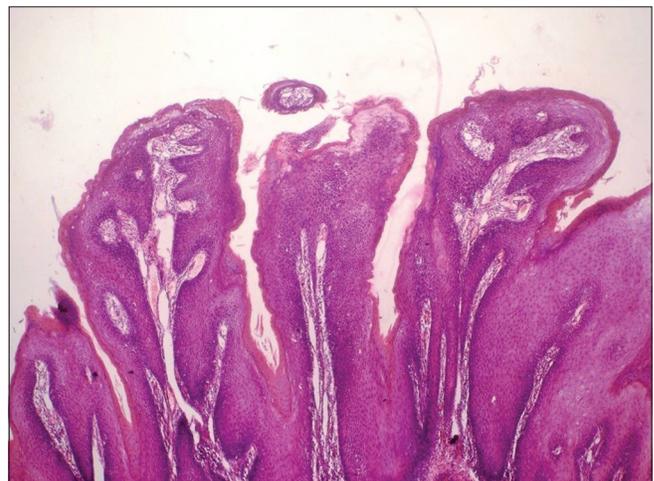


Figure 4: Photomicrograph of a verrucous hyperplasia. The verrucous growth is exophytic with blunt projections (H&E stain, $\times 100$)

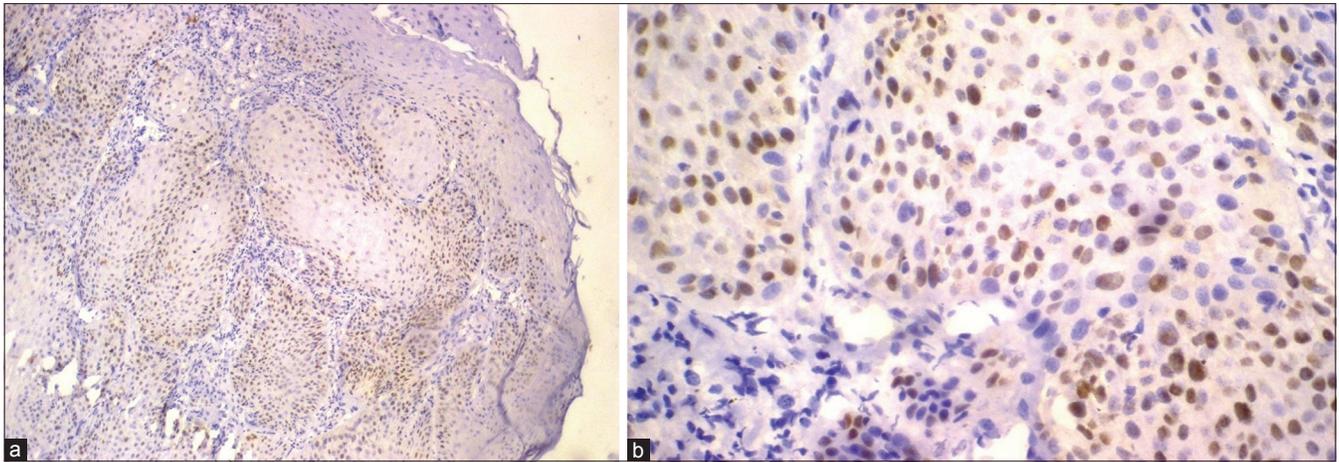


Figure 5: (a) Photomicrograph of oral verrucous carcinoma showing P53 positivity (IHC stain, $\times 100$). (b) Uniform p53 positivity in basal and parabasal layers of oral verrucous carcinoma. Intensive nuclear staining extends 3–10 cells above the basal cells in focal areas (IHC stain, $\times 400$)

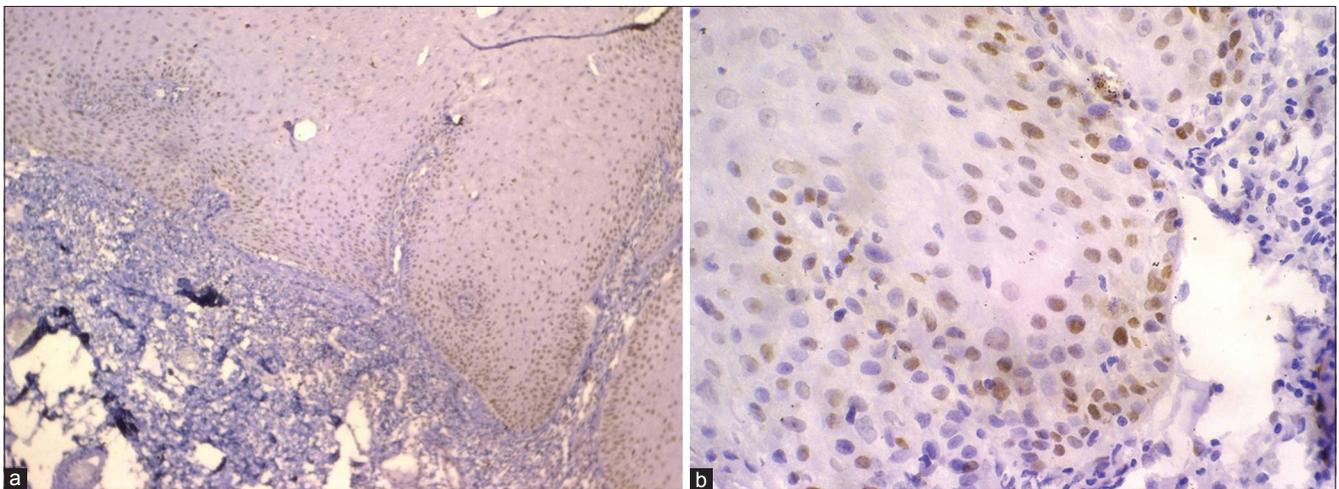


Figure 6: (a) Continuous p53 positivity observed in oral verrucous hyperplasia in basal and parabasal layers (IHC stain, $\times 100$). (b) Distribution of p53 protein in basal and parabasal layers of oral verrucous hyperplasia (IHC stain, $\times 400$)

with the application of Chi-square test. However, no statistical significance was found between the two verrucous lesions for p53 immunoreactivity ($P > 0.05$) [Table 3].

DISCUSSION

Very few studies have addressed the clinicopathologic enigma surrounding the two verrucous lesions, i.e. VH and VC, especially in South-East Asia, particularly in India. Our observations reveal that it is almost impossible to distinguish the two lesions clinically. Thus, the histopathologic differences between the two lesions act as a benchmark to arrive at a confirmatory diagnosis.

Shear and Pindborg^[3] defined OVH as a verrucous lesion in which the surface may be either sharp and heavily keratinized or blunt with a thin parakeratin layer. It is distinguishable from OVC only in biopsies taken at the margin of the lesion.

In OVC, the verrucous projections are superficial but the broad rete ridges extend much deeper than adjacent normal epithelium, with a pushing invasive border. Research studies have proved OVH as a precursor or an intermediate stage of OVC or OSCC formation.^[14,15]

The mean age of our patients with oral VC was significantly older than that of patients with oral VH, similar to findings by other studies.^[13,16] This suggests that oral VH may be a precursor lesion of oral VC.^[13] Shear and Pindborg^[3] also considered oral VH as a potentially precancerous lesion of oral VC. However, contrast observations were reported by other research studies where the elderly population was a more affected group in OVH.^[3,11,14]

Analyzing the demographic data, we observed a higher male preponderance in both these verrucous lesions simulating observations by the other authors in Taiwanese and Chinese population.^[13,14]

In contrast, elderly females were more commonly affected according to published literature on multifocal oral verrucous leukoplakia which shows histologic features of VH.^[3,16,17] Tobacco chewing was the predominant habit in VC as well as VH. In a study by Ghazali *et al.*,^[17] on VH group, and Walvekar *et al.*,^[18] on OVC, betel quid chewing was the most common habit with length of exposure to this habit reported from more than 10 years to more than 50 years. Nevertheless, cigarette smoking was more prevalent in Taiwan population^[12] and Chinese cohorts.^[1] In our study, the buccal mucosa was the most common site involved in both the lesions. However, these observations contrast with other research studies^[16,19] where gingiva and alveolar mucosa are most frequently involved, followed in order by the buccal mucosa, tongue, floor of the mouth, lip and palate. Similar contrast findings were reported by Shear and Pindborg^[3] on VH samples. The mean tumor size was larger in OVC when compared with OVH simulating the observations by Lin *et al.*^[13]

Epithelial hyperplasia with no atypia was seen in 22.3% of the cases with broad and blunt rete ridges in VH. Epithelial dysplasia was a prominent feature in 77.7% of the OVH cases. Similar observations were recorded in another study.^[3]

The p53 is not immunodetectable in normal epithelium; however, infrequently certain p53 gene mutations result in protein accumulation and hence become detectable. p53 mutation is not the only factor affecting p53 protein level. The immunoreactivity of p53 is also observed by the factors stabilizing p53 and the overexpression of p53 caused by DNA damage.^[8,9]

Klieb and Raphael^[20] investigated the expression of p53 in 32 oral VCs and 28 oral VH samples. They observed a more diffuse expression of p53 protein in oral VCs than that in oral VH lesions and suggested that IHC markers may be relevant as a diagnostic adjunct in difficult cases. However, our study showed no significant difference in p53 protein expression between oral VH and oral VC samples. Chang *et al.*^[21] found a consistent absence of p53 staining in their oral VC and oral VH samples. These differences can be attributed to using different samples, staining procedures and evaluation methods for the p53 protein.

As per the study by Lin *et al.*,^[13] their study suggested the failure of using the panel of IHC markers and partially explained the abnormal epithelial overgrowth in oral VC and oral VH lesions to MDM2 overexpression-induced inactivation of p53 protein. Thus, it is proposed that an incisional biopsy with adequate depth and the adjacent normal mucosa may serve as the gold standard for conclusive diagnosis of these two verrucous lesions. Because the individual cells are not very dysplastic, the pathologist must evaluate the overall histomorphologic

configuration of the lesion to arrive at an appropriate diagnosis. An adequate sampling is a must because as many as 20% of these lesions have a routine SCC developing concurrently within the VC.^[22] The close similarity in the expression and distribution pattern of p53 in the present study indicates that oral VH and oral VC are intimately related lesions showing a nearly similar degree of gene alterations. Therefore, early treatment of oral VH is of utmost consideration to prevent its malignant transformation to oral VC.

The oral VH and oral VC are closely related lesions which cannot be diagnosed clinically. Therefore, the diagnosis of oral VH and oral VC must be established histopathologically. Accurate histopathologic diagnosis depends on an adequate depth of the biopsy specimen and the adjacent normal epithelium. The present study suggests the inability of IHC marker like p53 protein to distinguish between these two verrucous lesions. Furthermore, this study was a clinicopathological analysis of patients with oral verrucous lesions, i.e., OVH and OVC, wherein no significant difference was found in the clinical parameters such as site, sex and habits while tumor size and age showed a statistically significant difference. Therefore, more series of research need to be done and a larger panel of IHC markers should be analyzed to dissolve the dilemma in differentiating these two lesions histopathologically.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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